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Biology of Symbioses Between Marine Invertebrates and Intracellular Bacteria

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Horst Felbeck

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The object of this research is to characterize symbiotic chemoautotrophic bacteria using molecular techniques. We are concentrating on the sequencing of their 16S rRNA to establish phylogenetic relationships between symbionts from different invertebrate hosts. We have also tested the use of oligonucleotide probes to identify putative symbionts in culture and are working to use this technique to detect ~~free living~~ symbionts. In addition, we compare genes for ribulose-1,5-bisphosphate-carboxylase and nitrogenase in a number of symbiotic systems using molecular probes of various origin. (Acc)

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ANNUAL PROGRESS REPORT ON CONTRACT N00014-88-K-0079

PRINCIPAL INVESTIGATOR: Horst Felbeck

CONTRACTOR: University of California

CONTRACT TITLE: Biology of Symbioses Between Marine Invertebrates and Intracellular Bacteria

START DATE: 1 January 1988

RESEARCH OBJECTIVE: To characterize symbiotic bacteria by 16S rRNA sequencing and to study their properties using oligonucleotide and gene probes

PROGRESS: During the initial phase of this contract we concentrated on two topics a) to test and develop methods for using oligonucleotiode probes and b) to characterize symbiotic bacteria based on information in their genome.

The postdoctoral researcher funded by this contract Dr. Daniel Distel achieved significant progress in methods development in the areas of sequence determination and use of oligonucleotide probes for symbiont detection and identification. He used as a model system the wood boring clam Lyrodus pedicellatus because, unlike in the case of the chemoautotrophic symbioses the bacterial symbionts have been cultured and the organism can be grown in the laboratory. He developed (in collaboration with Dr. Norman Pace's lab at Indiana University) oligonucleotide probes specific for this organism and used these to test the cultured bacteria proving that they indeed are the symbionts. These probes were fluorescently labeled and have been used on paraffin embedded and sectioned tissues. Probe specificity has been established using dot blot techniques against rRNA purified from a variety of closely related marine bacteria. Specific in situ labeling of bacterial cells mounted on glass slides has also been demonstrated.

We are currently working to decrease the detection limits and to enhance the resolution so that it may become possible to detect individual symbiont cells in tissues.

The second method we currently are trying to perfect is the specific polymerase chain reaction (PCR) amplification of symbiont sequences. Since most symbiont bacteria cannot be separated easily from their hosts, extracted nucleic acids are a mixture originating from host and bacteria. With PCR and specific procaryotic primers (directed against conserved regions in the16s rRNA sequences) it will be possible to use bulk nucleic acids from frozen bacteria containing tissues. It also will be possible to investigate symbionts from hosts which are very small or rare, e.g., the oligochaete worm Phallodrilus leukodermatus (weighing only 50 µg each), because areas of interest in the bacterial genome can be specifically amplified. Our results so far have been very encouraging, we were able to amplify specifically the DNA for 16s rRNA in a preparation of degraded DNA from bulk DNA of gills from a methane consuming bivalve. The fact that a degraded low molecular weight DNA could

be used for this purpose facilitates and widens the use of samples which have been badly preserved or have thawed during transports.

In parallel to the methods development we established a library of highly purified nucleic acids from a variety of symbiotic systems. We now have purified DNA of the animals listed in table 1 on hand, all of which can be digested with restriction enzymes. In addition, we obtained, tested and verified a number of gene probes for enzymes of CO_2 (ribulose-1,5-bisphosphate carboxylase; RuBisCo) and N_2 (nitrogenase) fixation (see table 2). Using these probes we could show with dot blots that in several symbiotic systems the gene for the enzyme nitrogenase (nif) is present (see table 3). This finding may have significant implications about the ways these symbioses obtain nitrogen for biosynthesis. With gene probes for the large and small subunits of RuBisCo we could establish relationships and homologies for this enzyme among different symbionts.

Table 1. Type and disposition of symbiont DNA samples to be characterized.

Organism	Location	Habitat Type
Tubeworms		
Riftia pachyptila	13° N. EPR	Hydrothermal Vent
Riftia pachyptila	Galapagos Rift	Hydrothermal Vent
Escarpia sp.	Gorda Ridge	Hydrothermal vent
Escarpia sp.	Juan de Fuca	Hydrothermal vent
Bivalves		
Bathymodiulus sp.	13° N. EPR	Hydrothermal Vent
Bathymodiulus sp.	Galapagos Rift	Hydrothermal Vent
Calyptogena magnifica	Galapagos Rift	Hydrothermal Vent
Calyptogena ponderosa	Louisiana Fan	Petroleum Seep
Pseudomiltha sp.	Louisiana Fan	Petroleum Seep
unident. mussel	Lousiana Fan	Petroleum Seep
unident. mussel	Florida Escarp.	Brine Seep
unident. mussel	Marianas Basin	Hydrothermal Vent
Gastropod		
Alvinochonca hessleri	Marianas Basin	Hydrothermal Vent

Table 2. Molecular probes available for this study.

Prokaryote	Type	Plasmid	Probe
Carbon Fixation (RuBisCo)			
Anabaena 7120	Cyanobacterium	An602	0.95 kb HpaI/HindIII
Anacystis nidulans	Cyanobacterium	pANP115	1.47 kb Pst/EcoRI
Xanthobacter sp. H4-14	Methylotroph	pUC9 w/pLL417R insert	0.7 Sma/Sal
Rhodospirillum rubrum	Photosynthetic	pRR2119	1.4 kb EcoRI/BglII
Rhodopseudomonas spaeroides	Photosynthetic	pRQ52 (II)	
Olisthodiscus leuteus	chromophytic alga	pOCPH 1.9	1.2 Kb EcoRI/BamHI
Nitrogen Fixation (Nif)			
Klebsiella pneumoniae	Enteric	pSA30	6.9 kb EcoRI

Table 3. Animal/bacteria symbioses with initial evidence for the presence of the nif gene

Organism	Location	Habitat Type
Alvinochonca hessleri	Marianas Basin	Hydrothermal vents
Lamellibrachia sp.	Louisiana Fan	Petroleum seep
unident. mussel	Louisiana Fan	Petroleum seep
Escarpia sp.	Juan de Fuca	Hydrothermal vents

WORK PLAN (YEAR 2):

The singular distinguishing feature in the obligate symbiosis of invertebrates with chemoautotrophic bacteria is their ability to fix carbon in the absence of sunlight with the oxidation of reduced sulfur or methane as an energy source. We plan to concentrate on the enzyme used by the sulfur oxidizing bacteria for this task, ribulose-1,5-bisphosphate carboxylase (RuBisCo) which is well characterized in higher plants and other bacteria, not, however, from any deep-sea bacteria. Since we have purified DNA of several symbionts on hand we plan to use the gene probes listed in table 2 to establish homologies of the large and the small subunit of this enzyme in the symbionts of different hosts. Depending on these results we will start to map this gene from the different symbiont species. The results of this study will then be compared to the phylogenetic tree obtained by 16S rNA seqeuncing.

We plan to expand our library of 16S rRNA sequences considerably during this year. We especially will focus on those of the symbionts of the protobranch clam Soleyma reidi, the methylotrophic mussel from the Louisiana oil seeps, and the gastropod Alvinochonca hessleri, and the comparative studies of symbionts of the same host species from widely separated areas.

We will also apply newly developed oligonucleotide probes to the symbioses with chemoautotrophic bacteria, to detect the bacteria in ambient water samples, to improve the sensitivity, and to test the specificity further.

PUBLICATIONS AND REPORTS (Year 1):

Felbeck, H., Distel, D., Stein, J.: Molecular Biology in Hydrothermal Vent Research. Presentation and Abstract, 5th Deep-Sea Biology Symposium, Brest/ France, June 26 - July 1, 1988. Invited talk

Felbeck, H.: Bacterial symbionts of Hydrothermal vent Organisms. 88th Annual Meeting of the American Society for Microbiology, May 8 -13, 1988. Invited talk

Haygood, M., Stein, J., Felbeck, H.: Molecular Approaches to Symbiosis Research. 88th Annual Meeting of the American Society for Microbiology, May 8 - 13, 1988. Invited talk

TRAINING ACTIVITIES

Research Assistantship for Graduate Students Jeff Stein and Tristan Darland (both part of the year) and salary for Postdoctoral Researcher Dr. Daniel Distel.